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13. ABSTRACT (Maximum 200 Words)

The goal of this research is to investigate the potential of (-)-epigallocatechin-3-gallate (EGCG), genistein and resveratrol, alone and in combination, to protect against prostate cancer in an animal model that spontaneously develops prostate cancer (TRAnsgenic Mouse Prostate adenocarcinoma (TRAMP)). The specific aims are 1) to investigate the potential of genistein, EGCG and resveratrol, alone and in combination, to suppress the development of spontaneously developing prostate tumors and 2) to investigate the potential of genistein, EGCG and resveratrol to regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanism of prostate cancer prevention. To date, we have demonstrated that pure resveratrol in the diet, but not EGCG in the water, suppressed spontaneously developing prostate tumors in TRAMPs. Androgen and estrogen receptors and EGF, IGF-1, and ERK signaling pathways are differentially regulated in the DLP of genistein, resveratrol and EGCG treated mice. We are in the process of investigating combinational genistein and resveratrol treatments to suppress prostate cancer in TRAMPs and to investigate mechanisms of action in mice treated with these 2 polyphenols.

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Introduction

Asians consuming a diet high in soy products have reduced incidence of clinically manifested prostate cancers. Likewise, Asians have a long history of drinking tea. Significant components of these two staples of the traditional Asian diet are the polyphenolic compounds. The primary polyphenols associated with prostate chemoprevention are the soy isoflavone, genistein, and the tea catechin, (-)-epigallocatechin-3-gallate (EGCG). Another polyphenol that has recently received attention as a cancer suppressor is resveratrol, a component of grapes. The goal of this research is to investigate the potential of these 3 pure polyphenols, alone and in combination, to protect against prostate cancer. In this manner, it may be possible to ingest moderate amount of each of these foods/chemicals, as opposed to mega amounts of one, and receive an additive or synergistic protective effect without adverse effects with possible elevated exposure.

Body

Aim 1) To investigate the potential of the polyphenols, genistein, EGCG and resveratrol, alone and in combination, to protect against prostate cancer. This is being evaluated in the TRAnsgenic Mouse Prostate adenocarcinoma (TRAMP) model that spontaneously develops prostate cancer.

Task 1, Phase I. Having already determined that genistein in the diet was able to suppress prostate cancer in TRAMP mice (previous work funded by NIH), the first phase of this Aim was to determine the potential of EGCG in the water and resveratrol in the diet, each alone, to suppress spontaneously developing prostate cancer in TRAMP mice. Relying on preliminary data from mammary chemoprevention studies with genistein, EGCG and resveratrol in female rats, where the low doses did not exert chemopreventive effects, we concentrated on determining if single exposure to the proposed high doses of EGCG (0.06% in drinking water), and resveratrol (625 mg/kg diet) starting at 5 weeks of age, would suppress prostate cancer development. C57BL/6 males and TRAMP females were bred and offspring were produced. The offspring were evaluated for transgene expression, and male TRAMP mice were subjected to these nutritional chemicals in water or AIN-76A diet, starting at 5 weeks postpartum. The groups contained 27 and 26 TRAMPs treated with EGCG and resveratrol, respectively, and 59 controls. The latter number is larger because we were able to utilize similarly treated control TRAMPs from another study. Necropsy was carried out when the animals were 28 weeks old or if animals became moribund. Histopathological evaluation of the tumors was carried out by Dr. Isam Eltoum, Board Certified Pathologist, using an established pathology scoring system for mice (1).

A summary of the histopathology report is found in Tables 1 and 2. Importantly, we found that the resveratrol treated group had a significantly smaller percentage of animals with poorly differentiated tumors (Score 6) as compared to controls (3.8% versus 20.3%, p-value = 0.045, Fisher's exact test), while the EGCG group was not significantly different than controls (18.5% versus 20.3%, p-value=0.547, Fisher's exact test). Also, the Resveratrol group had a significantly greater percentage of animals with well-differentiated tumors (Score 4) than controls (61.5% versus 37.3%, p-value=0.033, Fisher's exact test), while the EGCG group was not significantly different than controls (40.7% versus 37.3%, p-value=0.471, Fisher's exact test). These results demonstrate that pure resveratrol, and not pure EGCG, suppressed spontaneously developing prostate tumor development in this animal model as evidenced that resveratrol in the diet significantly reduced the incidence of poorly differentiated prostatic adenocarcinomas (Grade 6 tumors) and delayed the progression of well differentiated (Grade 4 tumors). EGCG treated animals did not demonstrate a significant change in histopathology when compared to the control animals.

Table 1

	Number of animals for each grade (1-6)						
Pathological Score	1	2	3	4	5	6	Total
Treatment							
Control	6	0	18	22	1	12	59
EGCG	2	0	8	11	1	5	27
Resveratrol	2	0	6	16	1	1	26

Table 2

	Percentage of animals for each grade (1-6)					
Pathological Score	1	2	3	4	5	6
Treatment						
Control	10.2	0	30.5	37.3	1.7	20.3
EGCG	7.4	0	29.6	40.7	3.7	18.5
Resveratrol	7.7	0	23.1	[redacted]	3.8	[redacted]

1 – No tumor; 2 – Low-grade PIN; 3 – High-grade PIN; 4 – Well-differentiated lesion; 5 – Moderately differentiated lesion; 6 – Poorly differentiated lesion. [a] < 0.05 compared to controls using Fisher's exact test. Significance was set at $p < 0.05$.

Body Weight and Food Consumption. There was not statistical difference for body weight and food and water consumption between treatment groups.

Ongoing/Future direction of Aim 1. Based on the data presented above and our original proposed Task 1 where we stated that we would investigate those nutritional agents that demonstrate efficacy, we will investigate the potential of resveratrol and genistein in combination, and not EGCG, to suppress spontaneously developing prostate tumors. Our previous work showed that 250 mg genistein/kg diet suppressed prostate cancer development (2) so we will use that dose and continue with the 625 mg resveratrol/kg diet used in the above reported work. Accordingly, the following groups of animals are being set up (Task 1, Phase II):
Group 1) Controls fed AIN-76A diet
Group 2) 250 mg Genistein/kg AIN-76A diet
Group 3) 625 mg Resveratrol/kg AIN-76A diet
Group 4) 250 mg Genistein + 625 mg Resveratrol/kg AIN-76A diet

Aim 2) To investigate the potential of genistein, EGCG and resveratrol to regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanisms of prostate cancer prevention. From the dorsolateral prostates of mice exposed ± polyphenols we will investigate expression of the androgen receptor (AR), estrogen receptors (ERs), epidermal growth factor (EGF) signaling, insulin-like growth factor-I (IGF-I) signaling, and extracellular signaling regulating kinases-1 and 2 (ERK-1 and ERK-2). (Months 18-36).

Male mice were exposed to the treatment diets starting at 5 weeks of age and sacrificed at 12 weeks of age. At dissection, the dorsolateral prostate (DLP) was excised. Western blot analysis and ELISA were employed to measure protein expression of sex steroid- and specific growth factor- receptors and ligands: We have completed the first phase of the mechanisms of action experiments using the following groups (at least 8 samples per group with 3 mice pooled per sample).

1) TRAMP males fed AIN-76A phytoestrogen-free powder diet (Controls)
2) TRAMP males fed 625 mg Resveratrol/kg Diet
3) TRAMP males given 0.06% EGCG in water
4) TRAMP males fed 250 mg Genistein/kg Diet

Summary Results

Resveratrol Treatment. In the DLP, resveratrol treatment significantly decreased IGF-1 and phospho-ERK 1, and significantly increased AR, ER-beta, IGF-R1, EGF-R, and EGF-1 protein expressions. ER-alpha, IGF-binding protein-3 (IGF-BP3), and total-ERKs 1 & 2 were not significantly different in resveratrol treated TRAMPs compared to control treated TRAMPs. The decrease in the potent growth factor, IGF-1 and down-stream phospho-ERKs and the increase in the prostate tumor suppressor, ER-beta, may provide a biochemical basis for resveratrol suppressing prostate cancer without significant toxicity. However, it is not clear why EGFR and EGF are upregulated, and yet resveratrol suppresses prostate cancer.

EGCG Treatment. In the DLP, EGCG treatment significantly decreased IGF-1, and significantly increased EGF-R, and EGF protein expressions. AR, ER-alpha, ER-beta, IGF-1R, IGF-BP3, total-ERKs 1 & 2, and phospho-ERKs 1 & 2 were not altered in EGCG treated TRAMPs compared to control TRAMPs. Perhaps the decreased IGF-1 levels and up-regulated EGF and EGFR cancel each other out in promoting prostate cancer.

Genistein Treatment. In the DLP, genistein significantly decreased ER-beta, IGF-1, IGF-BP3, EGF and EGFR protein expressions. AR, ER-alpha, IGF-1R, total-ERKs 1 & 2 and phospho-ERKs 1 & 2 were not significantly altered in genistein treated mice. These results confirm previous work from our lab (3).

Cell Proliferation. We used the PCNA (Proliferating Cell Nuclear Antigen) assay to investigate proliferation within the prostate. At 12 weeks of age, there was no significant change between EGCG or resveratrol treated animals and control mice.

Key Research Accomplishments

- Pure resveratrol in the diet suppressed spontaneously developing prostate tumors in TRAMPs.
- Resveratrol treatment significantly decreased IGF-1 and phospho-ERK 1 expression in the DLP.
- Resveratrol increased AR, ER-beta, IGF-R1, EGF-R, and EGF-1 protein expressions.
- ER-alpha, IGF-BP3, and total-ERKs 1 & 2 were not significantly different in resveratrol treated TRAMPs compared to control treated TRAMPs
- Pure EGCG in the diet did not alter development of prostate tumors in TRAMP mice.
- In the DLP, EGCG treatment significantly decreased IGF-1, and significantly increased EGF-R, and EGF protein expressions.
- AR, ER-alpha, ER-beta, IGF-1R, IGF-BP3, total-ERKs 1 & 2, and phospho-ERKs 1 & 2 were not altered in EGCG treated TRAMPs compared to control TRAMPs.
- Genistein significantly decreased ER-beta, IGF-1, IGF-BP3, EGF and EGFR protein expressions in the DLP.
- AR, ER-alpha, IGF-1R, total-ERKs 1 & 2 and phospho-ERKs 1 & 2 were not significantly altered in genistein treated mice.
- EGCG and resveratrol did not significantly alter cell proliferation in the DLP.

Reportable Outcomes

Wang, J and Lamartiniere, C.A. Genistein and estrogen regulation of androgen receptor, and EGF- and IGF-signaling in prostates of C57BL/6 mice. Proceedings of the AACR Annual Meeting. 2004.

Harper, C. E., Patel, B. B., Wang, J. and Lamartiniere, C. A. Resveratrol Action on Steroid and Growth Factor Signaling In TRAMP Mice. Proceedings of the Society of Toxicology. 2005.

Harper, C. E., Patel, B. B., Wang, J. and Lamartiniere, C. A. The Prostate Cancer Chemopreventive Effects of Resveratrol. Proceedings of the American Association for Cancer Research. 2005.

Conclusions

We conclude that pure resveratrol in the diet, but not pure EGCG in the water, suppresses spontaneously developing tumors in TRAMPs. Out of all of the sex steroid and growth factor signaling proteins measured, only the down-regulation of IGF-1 by resveratrol and genistein is consistent with the accepted dogma for chemoprevention. However, EGCG also down regulated IGF-1, but did not suppress prostate cancer in this model. It appears that protein levels of sex steroid and growth factor signaling proteins do not readily explain cancer control.

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Appendices

None